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Flumequine Distribution and Tissue Kinetics in Rainbow Trout (Salmo gairdneri Richardson). Detection of Residues

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In an earlier study (Michel et al, 1980), we demonstrated both the value of flumequine in treating furunculosis in salmonids and the absence of any signs of acute toxicity in the subjects treated.

Toxicity is an important factor in assessing potential risks for the consumer as a result of residues that may be present in the fish. As a guideline, oral LD50 values for flumequine range from 2,210 mg/kg to 2,450 mg/kg in rats and mice (Benothmane, 1979).

Half-life also indicates how the product behaves in the organism. In the case of flumequine, it is usually close to 6-7 hours in mammals, i.e., 5¼ hours in rats, 6½ hours in monkeys and 7 hours in humans (Lemeland and coworkers, 1981).

The purpose of this study was to detect flumequine residues in rainbow trout 6, 12, 24, 48, 72 and 96 hours after cessation of a 5-day treatment with 6 or 12 mg/kg/day. The compound was administered in the food.

Microbiological determinations of flumequine levels in target organs at different times provided a clear picture of the substance's distribution in the tissues as well as of the kinetics of elimination.

I. Materials and Methods

A. Experimental Procedure

Two homogeneous lots of 24 rainbow trout, weighing 120 g to 150 g each and maintained in water kept at 12°C ± 1°C, were administered flumequine in their food for five days. Dosage was as follows:

6 mg/kg flumequine for lot I

12 mg/kg flumequine for lot II

There was also a control, untreated lot.

Four animals were selected from each lot 6, 12, 24, 48, 72 and 96 hours after the last administration.

Blood was obtained by puncture of the dorsal aorta. The subjects were immediately deep-frozen for later blood and tissue determinations of flumequine levels.

B. Microbiological Determinations

The method of Chabbert and Boulingue (1957) was used. Calibrated muscle, liver, gut and gill fragments were cut up with the aid of a circular surgical knife and deposited on Mueller-Hinton agar which had previously been massively inoculated with a *Bacillus subtilis* spore culture (CIP 5262).

In order to facilitate diffusion between the tissue and agar, the fragments were inserted into tiny wells made in the agar by means of a punch roughly the diameter of the fragments.

A standard range of flumequine solutions containing 0.25, 0.50, 1.25, 2.5 3.75 and 5 µg flumequine was prepared. Under identical

conditions, 50 µl of each dose were introduced into wells made in the same agar plate which was then incubated at 30°C for 24 hours.

Determinations were based on a comparison between bacillus growth-inhibition zones around the tissue samples with inhibition zones surrounding the standard doses. In this particular case, minimal levels of roughly 0.1 µg could be detected in the samples deposited.

C. Spectrofluorimetric Determinations in Edible Muscle Tissue

The trout specimens were gutted and decapitated, then crushed and suspended in 400 ml demineralized water. 200 ml aliquots were clarified with 8 ml 3N perchloric acid, extracted with 450 ml chloroform in the presence of 25 ml 2N HCl, then poured into test tubes. Following centrifugation, the chloroform phase was collected on phase separation paper and evaporated by means of Rota Vapor equipment. The residue was dissolved in 10 ml chloroform. An equal volume of 0.1N NaOH was added. Following centrifugation, 8 ml of the aqueous phase, containing flumequine, were collected.

2 ml concentrated (25N) sulfuric acid were added prior to readings on the spectrofluorimeter against a 0.08N NaOH-5N H_2SO_4 control. The excitation wavelength was equal to 322 nm and the emission wavelength, to 385 nm.

A calibration curve was prepared in which fluorescence was expressed in terms of flumequine levels in the samples.

Since a linear relationship existed for doses of up to 200 µg flumequine per trout, the amount of flumequine present in each fish could be calculated from the following equation:

$$C = \frac{F-B}{m} \times D$$

Concentration C is expressed in µg active substance per trout, F equals fluorescence of the assay sample, B equals fluorescence of the flumequine-free control, m represents the slope of the reference curve (0.75 in this case) and D, a dilution factor (equal to 1 in this instance).

II. Results

A. Detection of Flumequine in Muscle, Liver, Digestive Tract and Gill Tissue by Means of Microbiological Tests

Fig. 1 shows the results of tissue kinetic studies following five days of treatment with 6 mg flumequine/kg. Fig. 2 gives the findings of treatment with 12 mg flumequine/kg.

In muscle tissue, it was noted that 6 hours after the last administration of 6 mg flumequine/kg for 5 days, tissue levels of the compound were still higher than minimum concentrations required to inhibit most susceptible organisms. These concentrations range from 0.2 to 1 µg/g (Rohlfing et al, 1977). After this period, flumequine levels approached the zero level.

Higher concentrations were recorded in the liver than in muscle tissue and remained bacteriologically active for 12 and 24 hours after cessation of treatment. The highest concentrations were recorded in the digestive tract, but as in the case of the liver, levels plummeted after 24 to 48 hours.

It was interesting to note that the substance still occurred in the gills for periods of up to 96 hours at levels above the minimum concentrations of inhibition recorded with most microorganisms.

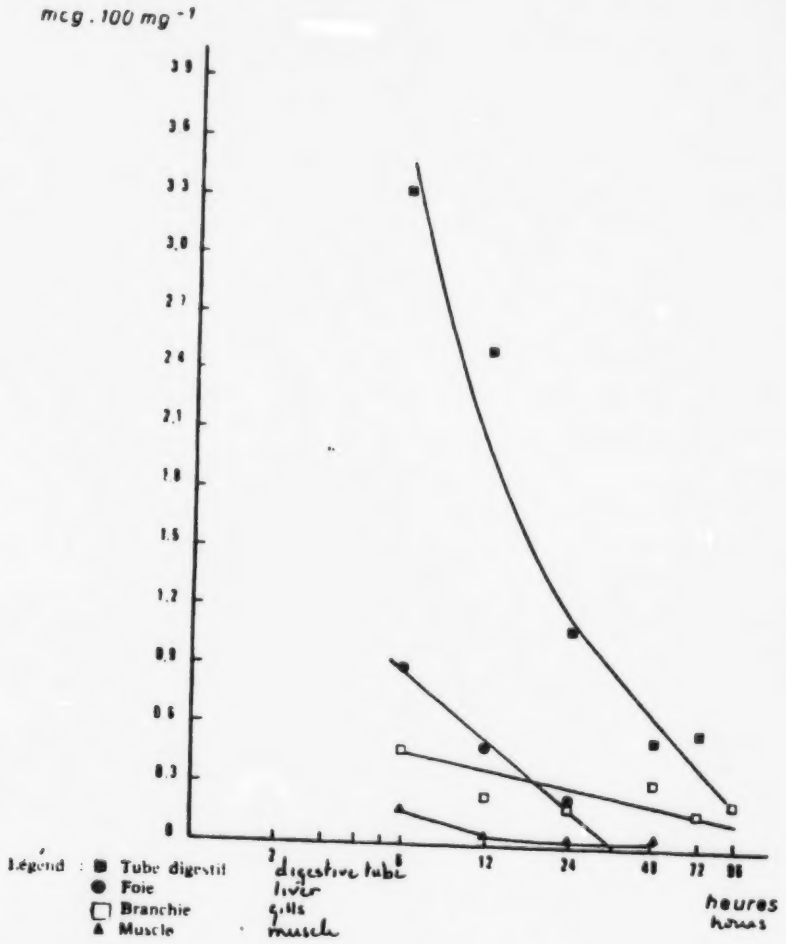

Fig. 1. Flumequine levels in the tissues as a function of time following oral treatment in trout given 6 mg/kg of body weight/day for 5 days

While findings with 12 mg flumequine essentially duplicated the results with 6 mg flumequine, certain differences became apparent.

Muscle levels were higher with 12 mg flumequine and were maintained for 24 hours. In the digestive tract, levels plateaued from 6 to 24 hours, then plunged, whereas with 6 mg/kg, there was a gradual downward slope.

When calculating mean six-hour values, we did not take animals nos. 2 and 4 into account since in neither animal could the compound be detected in the liver or, in the case of animal no. 2, in the muscles after 6 shours. Values recorded in the gills and digestive tract were very low compared to those found in the other individuals.

It may well be that these animals did not ingest the same amount of flumequine. There is a definite pecking order among trout and since the fish rapidly ingest the food distributed to them, some individuals may have received less or none at all.

B. Determination of Flumequine Levels in Muscle Tissue

Spectrofluorimetric results are shown in Table 3.

The technique is highly sensitive and substantiates our earlier findings. Muscle flumequine levels were either undetectable after 24 hours or else present in minimal amounts.

III. Discussion

With the experimental conditions we selected, it was found that in trout, five days of treatment with 6 or 12 mg/flumequine/kg of body weight had the following effect:

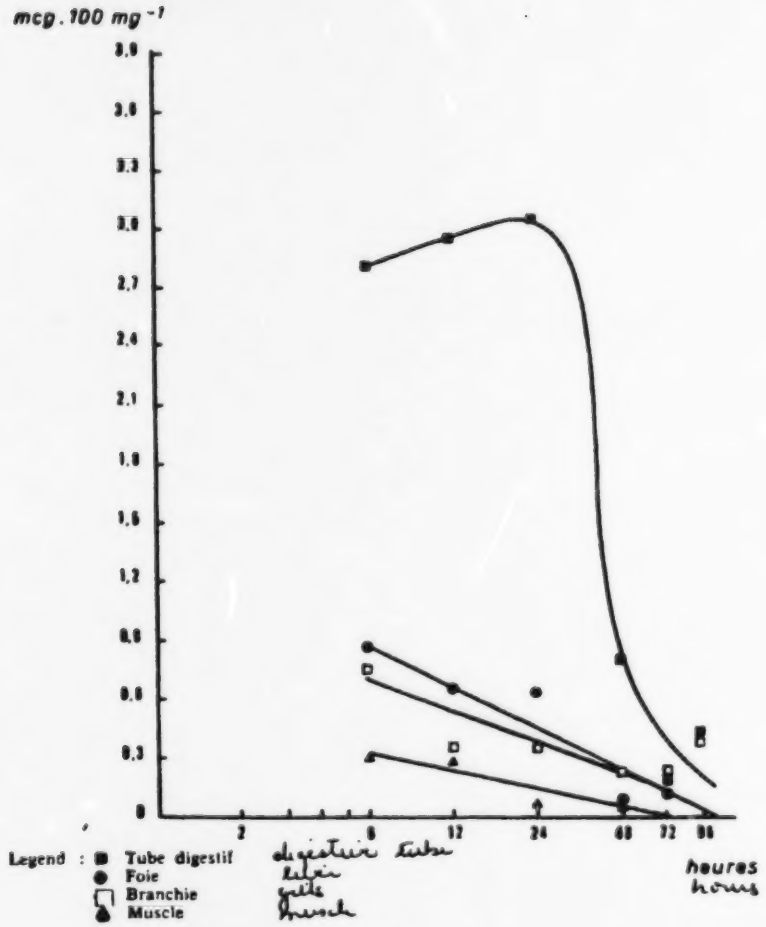

Fig. 2. Flumequine levels in the tissues as a function of time following oral treatment in trout given 12 mg/kg of body weight/day for 5 days

	LOT I - 6 mg/kg			LOT II - 12 mg/kg		
	Brut mcg/truite	ppm	Mean Moyenne	Brut mcg/truite	ppm	Mean Moyenne
6 heures	79,3 42,1 39,3 57,3	1,18 0,78 0,83 0,97	0,94	5,6 112,4 0 73,8	0,07 2,61 0 1,67	1,08
12 heures	73,6 15,9 32,5 53,1	0,89 0,27 0,75 0,60	0,62	157,9 142,7 62,8 90,3	2,67 2,59 1,30 1,61	2,04
24 heures	26,9 22,8 38,0 27,0	0,39 0,47 1,00 0,56	0,60	68,2 54,2 44,9 43,5	1,05 0,92 0,76 0,92	0,91
48 heures	1,3 0,0 0,0 0,0	0,02 0,00 0,00 0,00	0,00	19,3 0,0 0,5 0,0	0,32 0,00 0,00 0,00	0,08
72 heures	0,0 0,0 0,0 0,0	0,00 0,00 0,00 0,00	0,00	0,0 0,0 0,0 0,0	0,00 0,00 0,00 0,00	0,00
96 heures	0,0 0,0 0,0 0,0	0,00 0,00 0,00 0,00	0,00	0,0 0,0 0,0 0,0	0,00 0,00 0,00 0,00	0,00

Table 3. Spectrofluorimetric determination of flumequine levels in total edible muscle tissue

The compound disappeared rapidly from muscle tissue (after 12 or 24 hours).

Elimination was slightly slower in the case of the liver but virtually complete after 48 hours.

High levels were recorded in the digestive tract after 6, 12 and 24 hours. Thereafter, levels plunged although traces were still found after 96 hours.

Elimination was relatively slow in the gills although levels remained relatively low.

Persistence of the compound in the digestive tract may be attributed to the levels obtained as a result of oral administrations. Furthermore, the fairly high levels concentrated in the liver must be eliminated via the bile ducts into the intestine.

It is more difficult to account for the low but persistent levels in the gills. These organs play a far from negligible role in the excretion of waste products. There may exist an active mechanism contributing to capture of the molecule. The phenomenon warrants investigation insofar as gill infections, due to Myxobacteria, for instance, might constitute an indication for treatment with flumequine.

Our experimental findings indicate that flumequine treatment in trout does not leave residues in muscle tissue after 48 hours. As a result, there is no risk for the human consumer.

These findings in rainbow trout corroborate observations in other animal species, i.e., calves, swine, poultry. This in itself deserves mention since the same does not always apply to other anti-

bacterial agents that are commonly used in fish-breeding operations (Grangaud and coworkers, 1980).